Safety and immunogenicity of the adjunct therapeutic vaccine ID93 + GLA-SE in adults who have completed treatment for tuberculosis: a randomised, double-blind, placebo-controlled, phase 2a trial

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Summary

Background A therapeutic vaccine that prevents recurrent tuberculosis would be a major advance in the development of shorter treatment regimens. We aimed to assess the safety and immunogenicity of the ID93 + GLA-SE vaccine at various doses and injection schedules in patients with previously treated tuberculosis.

Methods This randomised, double-blind, placebo-controlled, phase 2a trial was conducted at three clinical sites near Cape Town, South Africa. Patients were recruited at local clinics after receiving 4 months of tuberculosis treatment, and screened for eligibility after providing written informed consent. Participants were aged 18–60 years, BCG-vaccinated, HIV-uninfected, and diagnosed with drug-sensitive pulmonary tuberculosis. Eligible patients had completed standard treatment for pulmonary tuberculosis in the past 28 days. Participants were enrolled after completing standard treatment and randomly assigned sequentially to receive vaccine or placebo in three cohorts: 2 μg intramuscular ID93 + 2 μg GLA-SE on days 0 and 56 (cohort 1); 10 μg ID93 + 2 μg GLA-SE on days 0 and 56 (cohort 2); 2 μg ID93 + 5 μg GLA-SE on days 0 and 56 and placebo on day 28 (cohort 3); 2 μg ID93 + 5 μg GLA-SE on days 0, 28, and 56 (cohort 3); or placebo on days 0 and 56 (cohorts 1 and 2), with the placebo group for cohort 3 receiving an additional injection on day 28. Randomisation was in a ratio of 3:1 for ID93 + GLA-SE and saline placebo in cohorts 1 and 2, and in a ratio of 3:3:1 for (2×) ID93 + GLA-SE, (3×) ID93 + GLA-SE, and placebo in cohort 3. The primary outcomes were safety and immunogenicity (vaccine-specific antibody response and T-cell response). For the safety outcome, participants were observed for 30 min after each injection, injection site reactions and systemic adverse events were monitored until day 84, and serious adverse events and adverse events of special interest were monitored for 6 months after the last injection. Vaccine-specific antibody responses were measured by serum ELISA, and T-cell responses after stimulation with vaccine antigens were measured in cryopreserved peripheral blood mononuclear cells specimens using intracellular cytokine staining followed by flow cytometry. This study is registered with ClinicalTrials.gov, number NCT02465216.

Findings Between June 17, 2015, and May 30, 2016, we assessed 177 patients for inclusion. 61 eligible patients were randomly assigned to receive: saline placebo (n=5) or (2×) 2 μg ID93 + 2 μg GLA-SE (n=15) on days 0 and 56 (cohort 1); saline placebo (n=2) or (2×) 10 μg ID93 + 2 μg GLA-SE (n=5) on days 0 and 56 (cohort 2); saline placebo (n=5) on days 0, 28 and 56, or 2 μg ID93 + 5 μg GLA-SE (n=15) on days 0 and 56 and placebo injection on day 28, or (3×) 2 μg ID93 + 5 μg GLA-SE (n=14) on days 0, 28, and 56 (cohort 3). ID93 + GLA-SE induced robust and durable antibody responses and specific, polyfunctional CD4 T-cell responses to vaccine antigens. Two injections of the 2 μg ID93 + 5 μg GLA-SE dose induced antigen-specific IgG and CD4 T-cell responses that were significantly higher than those with placebo and persisted for the 6-month study duration. Mild to moderate injection site pain was reported after vaccination across all dose combinations, and induration and erythema in patients given 2 μg ID93 + 5 μg GLA-SE in two or three doses. One participant had grade 3 erythema and induration at the injection site. No vaccine-related serious adverse events were observed.

Interpretation Vaccination with ID93 + GLA-SE was safe and immunogenic for all tested regimens. These data support further evaluation of ID93 + GLA-SE in therapeutic vaccination strategies to improve tuberculosis treatment outcomes.

Funding Wellcome Trust (102028/Z/13/Z).

Lancet Respir Med 2021; 9: 373–86

Published Online December 8, 2020 https://doi.org/10.1016/S2213-2600(20)30319-2

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See Online for appendix

Research in context

Evidence before this study

Tuberculosis kills more people worldwide than any other infectious pathogen. The standard treatment regimen is lengthy, and disease recurs in 2–9% of patients. An effective adjunct tuberculosis vaccine that prevents disease recurrence would facilitate development of shorter and simpler treatment regimens. We searched PubMed using the terms "TB vaccine", "adjuvant", "subunit", and "clinical trial", with no restrictions on our search dates or language up to Sept 27, 2019. Several publications reported studies of killed mycobacterial preparations administered during tuberculosis treatment to improve end of treatment outcomes. One study reported on the use of a subunit vaccine M72/AS01$_E$ in tuberculosis-naive adults, adults previously treated for tuberculosis, and adults with tuberculosis who had completed the intensive phase of tuberculosis treatment. This vaccine was immunogenic but resulted in high local reactogenicity in adults undergoing tuberculosis treatment. We aimed to assess the safety and immunogenicity of the ID93 + GLA-SE vaccine at various doses and injection schedules in patients with previously treated tuberculosis.

Added value of this study

ID93 + GLA-SE vaccination of patients who had successfully completed standard treatment for tuberculosis was feasible, safe, and induced robust antigen-specific systemic cellular and humoral immune responses.

Implications of all the available evidence

Our trial is the first report of a subunit candidate tuberculosis vaccine delivered by the intramuscular route in multiple dose combinations to patients with tuberculosis upon completion of treatment. This study supports progression of ID93 + GLA-SE towards safety and efficacy trials of therapeutic vaccination during tuberculosis treatment to improve treatment outcomes and reduce disease recurrence.

Introduction

Tuberculosis disease is a major cause of morbidity, and the world's largest single infectious disease killer.[1] Drug-sensitive tuberculosis can be treated, but 2–9% of patients develop recurrent tuberculosis disease after treatment completion.[2–5] A systematic review[4] of 15 trials showed a maximum risk of recurrence within 12 months after completion of treatment. Development of shorter drug regimens for drug-sensitive tuberculosis has been limited by adverse outcomes, including recurrent disease.[6–8] Treatment of drug-resistant tuberculosis is characterised by high rates of treatment failure and associated mortality,[9,10] but as immediate treatment outcomes improve,[11] further simplification and shortening of any novel tuberculosis regimens will probably also be limited by post-treatment recurrence.

Prevention of recurrent tuberculosis would reduce the clinical, social, and financial burden of retreatment for patients, health workers, and national tuberculosis control programmes; reduce the risk of drug-resistant tuberculosis arising from inadequate therapy; and facilitate development of shorter therapeutic regimens for drug-resistant and drug-sensitive tuberculosis.[12] Development of an effective post-treatment vaccine to boost or redirect the host immune response to control reactivation of viable *Mycobacterium tuberculosis* might also protect against disease arising from re-infection. However, given the marked immunological perturbations associated with tuberculosis disease, which can persist for years after treatment completion,[13] it is not known whether post-treatment vaccination could modulate adaptive immune responses to afford long-term protection against recurrence. A proof-of-concept study[14] showed efficacy for the M72/AS01$_E$ vaccine in preventing tuberculosis disease among adults with immune sensitisation to *M tuberculosis* detected by QuantiFERON. These results support the hypothesis that a protein-plus-adjuvant vaccine might provide protection against recurrent disease in people with previously treated tuberculosis.[14]

Vaccination of patients with tuberculosis poses safety concerns, including the potential for large injection site reactions, as reported in the study of subunit vaccine M72/AS01$_E$.[15] Moreoever, a high bar for protective immunity might exist, given the influence of tuberculosis disease on the host immune response.[16] A vaccine could be administered during therapy, an approach for which the reports of therapeutic benefit of a multidose *M vaccae* vaccine provide some support.[17] However, the safety, immunogenicity, and optimal regimen for any candidate vaccine should first be shown upon completion of treatment when these risks are minimised, compared with earlier in treatment.

ID93 + GLA-SE was developed for prevention of tuberculosis disease in people infected with *M tuberculosis*, and as an adjunctive therapeutic vaccine to improve treatment outcomes. ID93 is a polyprotein comprised of four *M tuberculosis* antigens (Rv1813c, Rv2608, Rv3619c, and Rv3620c),[18] formulated with GLA-SE adjuvant, a synthetic toll-like receptor 4 agonist in a stable oil-in-water emulsion.[19] In murine studies, ID93 + GLA-SE induced robust polyfunctional Th1 responses and showed protection against challenge with multidrug-resistant tuberculosis.[20,21] ID93 + GLA-SE reduced treatment duration and improved outcomes in mice and non-human primates, compared with antibiotics alone.[22] The vaccine was then tested in non-endemic regions among people not infected with *M tuberculosis*, followed by testing in endemic regions. ID93 + GLA-SE had an acceptable safety profile in adults not infected with *M tuberculosis* in the USA[23] and in BCG-vaccinated adults with and without *M tuberculosis* infection in South Africa,

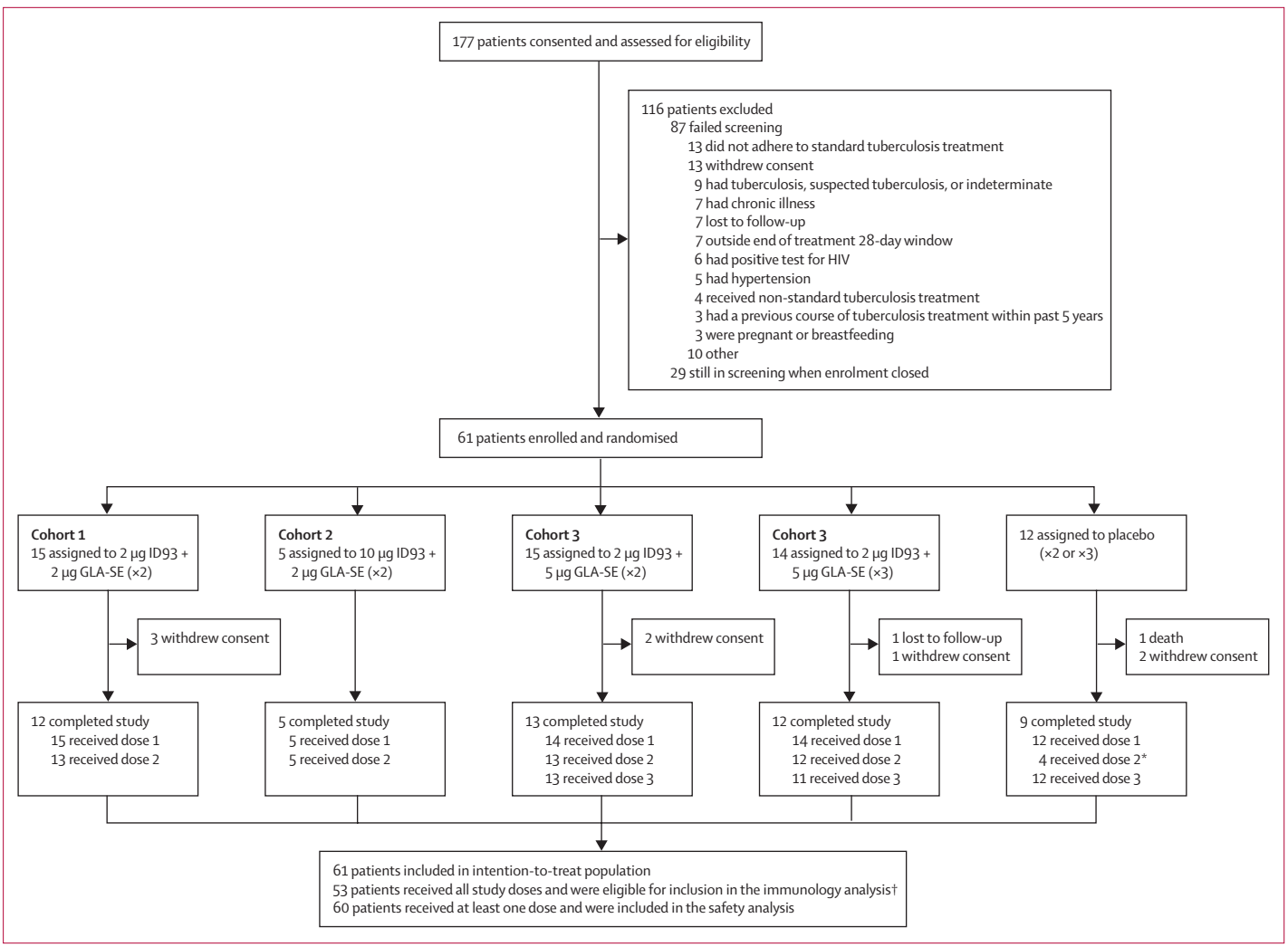


Figure 1: **Trial profile**
*Five participants were scheduled to receive placebo at day 28. The remaining placebo group participants were scheduled to receive injections at days 0 and 56 only. †Not all samples were available at all timepoints.

in whom long-lasting, antigen-specific CD4 T-cell and antibody responses were induced.[22] We report safety, reactogenicity, and immunogenicity for ID93 + GLA-SE administered at different doses and injection schedules in patients with tuberculosis after completion of standard treatment.

Methods

Study design and participants

This randomised, double-blind, placebo-controlled, phase 2 trial enrolled BCG-vaccinated, HIV-uninfected patients with drug-sensitive tuberculosis who were receiving standard treatment. Patients were recruited from local clinics and the study was conducted at three clinical centres near Cape Town, South Africa: the South African Tuberculosis Vaccine Initiative (SATVI), the TASK Clinical Research Centre, and the Desmond Tutu HIV Centre. Immunology samples were processed at SATVI and Stellenbosch University Immunology Research Group. The study protocol (appendix p 19) and informed consent form were approved by the Medicines Control Council of South Africa, the US Food and Drug Administration (FDA), and the Human Research Ethics Committees of the University of Cape Town and Stellenbosch.

Patients were recruited at local clinics after receiving 4 months of tuberculosis treatment, and screened for eligibility after providing written informed consent. Treatment lasted approximately 6 months, to a maximum of 28 weeks. Participants were aged 18–60 years, BCG-vaccinated, HIV-uninfected, diagnosed with drug-sensitive pulmonary tuberculosis, and had received standard treatment. Exclusion criteria included drug-resistant tuberculosis, severe abnormal laboratory results, use of immunosuppressive drugs within the past 6 months, or evidence of chronic illness. For a complete

	Enrolled (ITT population) (n=61)	Safety population (n=60)	Per protocol (completed) population (n=53)†	Study injections	ID93 dose	GLA-SE dose	Dosing schedule
Cohort 1	15	15	13	ID93 + GLA-SE	2 μg	2 μg	Days 0, 56
Cohort 2	5	5	5	ID93 + GLA-SE	10 μg	2 μg	Days 0, 56
Cohort 3	15	14*	13	ID93 + GLA-SE + placebo (saline)‡	2 μg	5 μg	Days 0,28 ‡, 56
Cohort 3	14	14	11	ID93 + GLA-SE	2 μg	5 μg	Days 0, 28, 56
Placebo	12	12	11	Placebo (saline)	..	..	Days 0, (28)‡, 56

*One patient was enrolled but never received a study vaccine. †Not all samples were available at all timepoints. ‡Saline placebo injection administered at day 28 to retain blinding in cohort 3.

Table 1: **Dose cohorts and treatment regimens**

listing of inclusion and exclusion criteria, see appendix (p 2).

Adherence to tuberculosis treatment was monitored by local clinic staff. Two sputum samples were obtained at around months 4, 5, and 6 (end of treatment) to confirm Xpert MTB/RIF or liquid culture negative for *M tuberculosis*. Tuberculosis treatment success was confirmed by negative sputum Xpert MTB/RIF assay, or by negative liquid culture on two successive occasions at least 30 days apart at approximately 4 and 5 months after the start of treatment. Participants with bacteriological confirmation of cure were eligible to participate in the study, and the first vaccine administration occurred within 28 days of the last dose of tuberculosis treatment.

Randomisation and masking

Eligible patients were enrolled sequentially into one of three cohorts and randomly assigned to receive vaccine or saline placebo in a schedule of two or three injections. Dose selection for all cohorts was informed by previous data from BCG-vaccinated adults with and without *M tuberculosis* infection (NCT01927159). Investigators, participants, outcomes assessors, and laboratory staff were masked to treatment. To maintain masking of the team at the study sites, the vaccine manager was a designated study team member, usually the study pharmacist, who had no clinical or regulatory responsibilities associated with the conduct of the study during the entire study period, other than managing the vaccine. Participants in cohort 3 assigned to ID93 + GLA-SE at days 0 and 56 received a saline study injection at day 28. The injection administrator was not masked, as placebo and study vaccine had different appearances, but they were not involved in assessment of outcomes. For detailed randomisation and masking methods, see appendix (p 1).

Procedures

Patients were assigned (3:1 cohort 1; 3:1 cohort 2; 3:3:1 cohort 3) to receive intramuscular injections of the study vaccine or placebo, as follows: 2 μg ID93 + 2 μg GLA-SE or saline placebo on days 0 and 56 (cohort 1, 3:1); 10 μg ID93 + 2 μg GLA-SE or saline placebo on days 0 and 56 (cohort 2, 3:1); or 2 μg ID93 + 5 μg GLA-SE on days 0 and 56 with placebo on day 28, 2 μg ID93 + 5 μg GLA-SE on days 0, 28, and 56, or saline placebo on days 0, 28, and 56 (cohort 3); (figure 1, table 1).

Participants were sequentially enrolled into subsequent cohorts, pending favourable safety review at day 7 of the previous cohort. Complete details on qualification for dose escalation to the next dose cohort are included in the appendix (p 83). The protocol was amended on Jan 14, 2016, to expand cohort 3 to include the 2 μg ID93 + 5 μg GLA-SE dose given as three injections.

Immunogenicity analyses were done on all participants who received all study injections, using samples obtained on study days 0, 14, 28, 56, 70, and 224, with an additional day 42 sample in cohort 3. Whole blood analyses were also included on day 84.

Vaccine-specific antibody responses, assessed as end-point titres of ID93-specific total IgG and subclasses (IgG1, IgG2, IgG3, and IgG4), were measured by serum ELISA. Patients in cohort 1 were further tested using individual ID93 subunit antigens (Rv1813, Rv2608, Rv3619, and Rv3620). For detailed ELISA methods, see appendix (p 3).

Antigen-specific T-cell responses were measured in cryopreserved cellular specimens using intracellular cytokine staining (ICS) followed by flow cytometry, after stimulation with vaccine antigens. T-cell responses from cryopreserved peripheral blood mononuclear cells (PBMC) and whole blood were assessed by ICS, followed by flow cytometry after 12 h stimulation with ID93 protein, or pools of overlapping 15-mer peptides representing the individual component antigens (Rv1813, Rv2608, Rv3619, and Rv3620 [Biosynthesis]). The PBMC stimulations included a megapool of 260 *M tuberculosis*-derived epitopes (MTB300), from which ID93-derived epitopes were omitted. Staphylococcal enterotoxin B stimulation was used as the positive control and peptide diluent was used as the negative control.

Whole blood ICS was done as previously described,[24] by stimulating with ID93 or peptide pools of the individual antigens for 12 h. Phytohemagglutinin (Bioweb) or media alone were used as controls. Frequencies of cytokine producing CD4 or CD8 T-cells were quantitated. For method details, see appendix (pp 3–6).

In the assessment of safety outcomes, participants were observed for 30 min after injection, and specific safety evaluations were done 3 and 7 days after each dose. Monitoring and assessment of injection site reactions and systemic adverse events (AEs) was done from day 0 to day 84. Serious adverse events (SAEs) and AEs of special interest were monitored for 6 months after administration of last vaccine. AEs were graded by severity (by use of the US FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials [2007]) and relationship to treatment by an investigator. Blood samples were obtained at baseline (screening) and 7 days after each injection (day 7 and 63 for all groups and day 35 for

	2 μg ID93 + 2 μg GLA-SE (×2; cohort 1, n=15)	10 μg ID93 + 2 μg GLA-SE (×2; cohort 2, n=5)	2 μg ID93 + 5 μg GLA-SE* (×2; cohort 3, n=14)	2 μg ID93 + 5 μg GLA-SE (×3; cohort 3, n=14)	Placebo† (cohorts 1–3, n=12)
Age, years	27·0 (18·0–54·0)	32·0 (18·0–43·0)	23·5 (18·0–41·0)	27·0 (19·0–36·0)	27·0 (21·0 –50·0)
Sex					
Female	11 (73%)	3 (60%)	7 (50%)	2 (14%)	4 (33%)
Male	4 (27%)	2 (40%)	7 (50%)	12 (86%)	8 (67%)
Ethnicity					
Cape mixed ancestry	10 (67%)	1 (20%)	11 (79%)	7 (50%)	8 (67%)
Black African	5 (33%)	4 (80%)	3 (21%)	7 (50%)	4 (33%)
White	0	0	0	0	0
Body-mass index (kg/m²)	20·0 (18·0–26·0)	21·0 (19·0–32·0)	19·5 (17·0–25·0)	20·5 (17·0–37·0)	21·5 (18·0–25·0)

Data are median (min–max) or n (%). *Cohort 3 (2 × vaccine dose) received 1 saline placebo injection on day 28. †Saline placebo injections administered on days 0 and 56 (cohort 1 and cohort 2), or on days 0, 28, and 56 (cohort 3).

Table 2: **Patient baseline characteristics (safety population)**

	2 μg ID93 + 2 μg GLA-SE (×2; cohort 1, n=15)	10 μg ID93 + 2 μg GLA-SE (×2; cohort 2, n=5)	2 μg ID93 + 5 μg GLA-SE* (×2; cohort 3, n=14)	2 μg ID93 + 5 μg GLA-SE (×3; cohort 3, n=14)	Placebo† (cohorts 1–3, n=12)	p value‡	p value§
Any adverse event	12 (80%)	5 (100%)	13 (93%)	12 (86%)	9 (75%)	0·518	0·475
Grade 1	10 (67%)	5 (100%)	12 (86%)	12 (86%)	9 (75%)	0·459	0·765
Grade 2	4 (27%)	1 (20%)	6 (43%)	4 (29%)	5 (42%)	0·728	0·773
Grade 3	1 (7%)	0	0	2 (14%)	1 (8%)	0·797	0·504
Grade 4	0	0	0	0	0	1·000	1·000
Injection related reactions							
Injection site erythema	0	0	4 (29%)	1 (7%)	0	0·021	0·107
Injection site induration	1 (7%)	0	3 (21%)	3 (21%)	0	0·327	0·242
Injection site pain	5 (33%)	4 (80%)	10 (71%)	7 (50%)	3 (25%)	0·030	0·067
Laboratory investigations							
Alanine aminotransferase increased	2 (13%)	0	1 (7%)	3 (21%)	2 (17%)	0·916	0·650
Blood bilirubin increased	1 (7%)	0	1 (7%)	0	1 (8%)	1·000	0·749
Haemoglobin decreased	1 (7%)	1 (20%)	1 (7%)	0	0	0·492	1·000
White blood cell count decreased	0	1 (20%)	2 (14%)	2 (14%)	0	0·148	0·524
White blood cell count increased	1 (7%)	0	1 (7%)	1 (7%)	1 (8%)	1·000	1·000
General adverse events							
Headache	1 (7%)	2 (40%)	1 (7%)	0	3 (25%)	0·182	0·095
Hypertension	1 (7%)	0	1 (7%)	1 (7%)	0	1·000	1·000
Nasal congestion	0	1 (20%)	2 (14%)	0	0	0·148	0·318
Tonsillitis	0	0	3 (21%)	0	0	0·088	0·096
Upper respiratory tract infection	3 (20%)	1 (20%)	3 (21%)	1 (7%)	3 (25%)	1·000	0·582
Arthralgia	0	0	1 (7%)	0	0	0·674	1·000
Fatigue	0	0	0	1 (7%)	0	1·000	1·000
Myalgia	1 (7%)	0	0	0	0	1·000	1·000

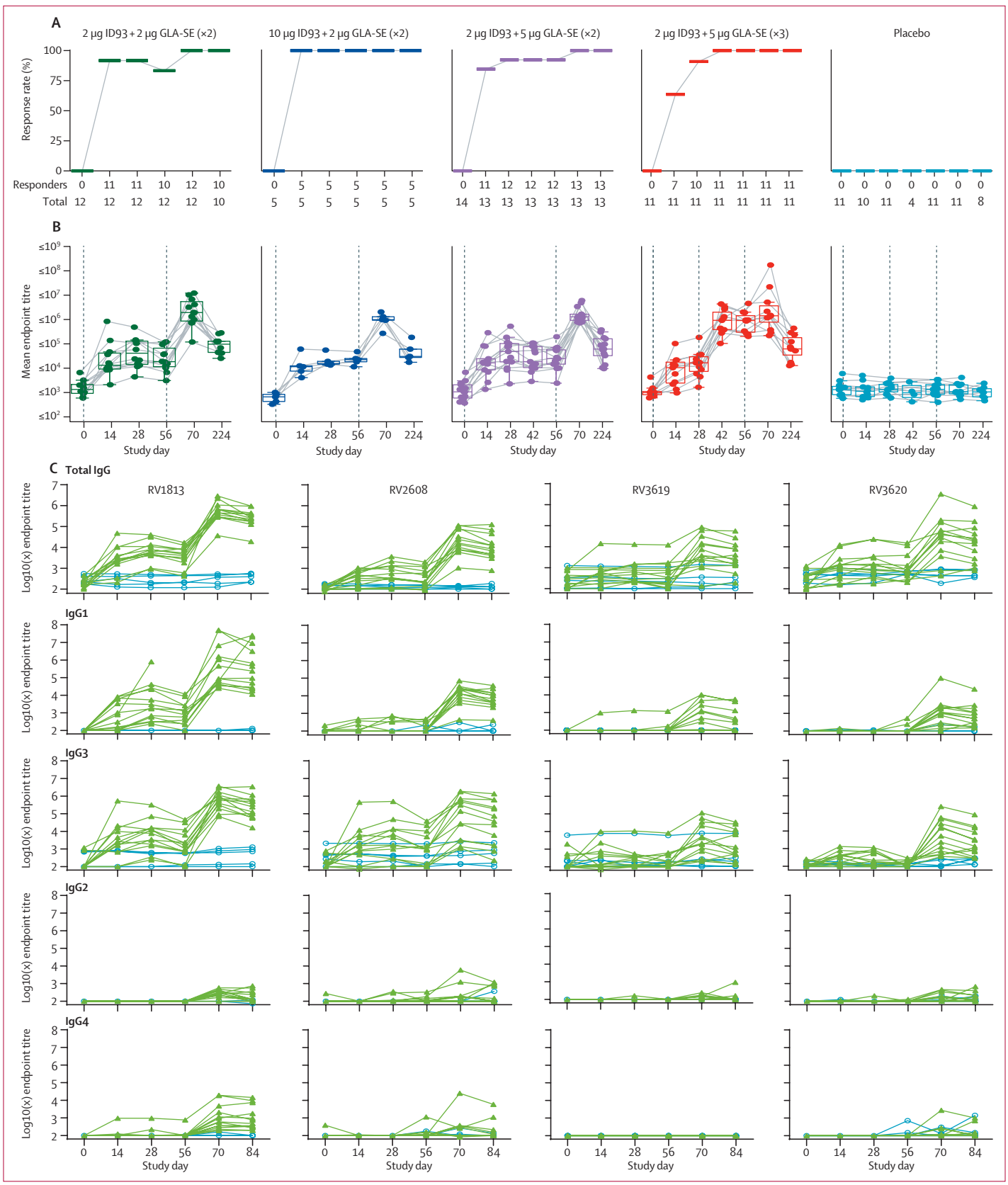
All p values obtained by Fisher's exact test. If significant (p<0·05), pairwise comparisons were performed with Bonferroni adjustments. *Cohort 3 (2 × vaccine dose) received 1 saline placebo injection on day 28. †Saline placebo injections administered on days 0 and 56 (cohort 1 and cohort 2), or on days 0, 28, and 56 (cohort 3). ‡p value for comparison of 2 μg ID93 + 2 μg GLA-SE (× 2), 10 μg ID93 + 2 μg GLA-SE (× 2), and 2 μg ID93 + 5 μg GLA-SE (× 2), versus placebo (× 2) groups at day 0–27 and 56–63 as appropriate (comparison of all two dose schedules *vs* 2 doses of placebo group). §p value for comparison of 2 μg ID93 + 5 μg GLA-SE (× 2), and 2 μg ID93 + 5 μg GLA-SE (× 3), versus placebo groups at day 0–27 and 56–63 as appropriate (comparison of all of cohort 3 vaccine group vs all of cohort 3 placebo population).

Table 3: **Adverse events by safety population**

cohort 3 only). Safety laboratory results were reviewed for any abnormality and repeated if necessary before the next scheduled injection.

Outcomes

The primary outcomes were safety (injection site reactions, systemic AEs, SAEs, and AEs of special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10 μg ID93 + 2 μg GLA-SE (×2)
2 μg ID93 + 5 μg GLA-SE (×2)
2 μg ID93 + 5 μg GLA-SE (×3)
Placebo
Response rate (%)
Responders
Total
B
Mean endpoint titre
Study day
C Total IgG
RV1813
RV2608
RV3619
RV3620
IgG1
IgG3
IgG2
IgG4
Log10(x) endpoint titre

interest) and immunogenicity (antigen-specific IgG antibody titres in serum as measured by ELISA and antigen specific T-cell frequencies in PBMC measured by ICS assay) of ID93+GLA-SE. Secondary immunogenicity outcomes included T-cell responses measured by whole blood ICS assay. The secondary objective was to assess whether gene expression signatures can predict IgG antibody and T-cell responses to ID93+GLA-SE after successful treatment of tuberculosis, and identify correlates of immunogenicity for ID93 + GLA-SE. This secondary objective allowed for banking of specimens for future analysis.

Statistical analysis

The sample size was designed to detect common AEs or patterns of AEs and was not powered to detect infrequent AEs. The assumed true AE probability was 2%, therefore with a sample size of 48 patients receiving ID93+GLA-SE there was estimated 95% probability to detect any severe AE or SAE. If no severe AE or SAE were observed, the upper limit of the 95% CI for the true probability for such an event was 7·9%. Descriptive summaries with proportions were presented for safety outcomes and participant characteristics. Fisher's Exact tests were used to analyse categorical data at the 0·05 significance level with Bonferroni adjustment for multiple pairwise comparison. Safety was evaluated by comparing proportions of participants with local and systemic AEs, including grade, and SAEs in the three ID93+GLA-SE cohorts in the safety population (all patients who received at least one study injection) with the total placebo group.

Immunogenicity analyses were prespecified in a statistical analysis plan to analyse the difference in the immune responses elicited by two versus three doses of 2 µg ID93+5 µg GLA-SE (cohort 3); the difference in the immune response elicited by (2×) 2 µg ID93+2 µg GLA-SE (cohort 1), (2×) 10 µg ID93 +2 µg GLA-SE (cohort 2), and (2×) 2 µg ID93 +5 µg GLA-SE (cohort 3); and the difference in immune response elicited by each of the four ID93 regimens versus placebo. Immunogenicity outcomes were assessed in participants who received all study doses as specified for the assigned treatment group in the protocol at the time of sample collection. Data were not available for analysis at all timepoints due to missing samples or technical issues. ELISA-positive response criteria were defined as a four-times increase from baseline. Antigen-specific T-cell responses were based on CD4 T cells expressing at least two cytokines. T-cell response magnitude was calculated by subtracting the unstimulated response from the antigen-stimulated response. T-cell response positivity was calculated using MIMOSA,[25] a Bayesian statistical framework that controls the false-discovery rate to 0·1%. Summary statistics, boxplots, and hypothesis tests were computed with both responders and non-responders combined. For further details, see appendix (p 6).

Continuous responses in two treatment groups were compared using the Wilcoxon rank-sum test. Response rates were compared using Fisher's exact test. Significance was based on a two-sided test with $p<0·05$. When multiple groups were considered, continuous responses were compared across treatment groups using the Kruskal-Wallis test. Binary response rates were compared across treatment groups using an $n \times m$ Fisher's exact test. To adjust for multiple group comparisons, we applied the Holm-Bonferroni method to control the family-wise error rate (FWER); comparisons with FWER-adjusted $p<0·05$ were considered to be significant. Statistical analyses for humoral, whole blood ICS, and PBMC ICS were done using identical methods.

PBMC ICS data were also analysed using COMPASS (combinatorial polyfunctionality analysis of antigen-specific T-cell subsets).[26] COMPASS uses a Bayesian hierarchical framework to model all observed functional cell subsets and select those most likely to show antigen-specific responses. Cell subset responses were quantified by posterior probabilities for responses. Cell subsets that did not have at least five cells in at least two participants were excluded.

This study is registered with ClinicalTrials.gov, number NCT02465216.

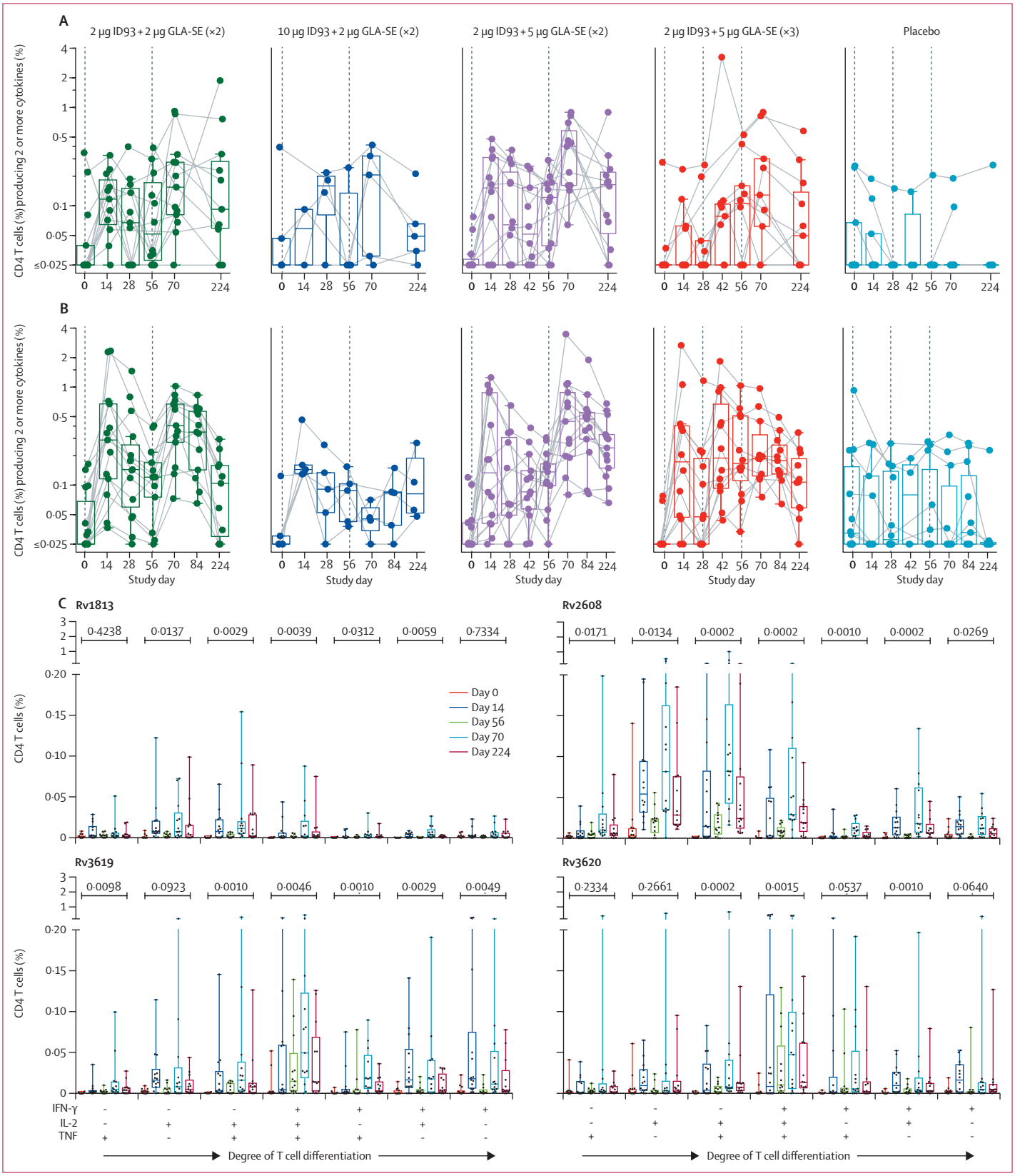
Role of the funding source

The funder of the study (Wellcome Trust) had a role in study design and interpretation of the data. The sponsor of the study (Infectious Disease Research Institute) had a role in study design, data interpretation, data analysis, and writing of the report, but not in data collection. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 17, 2015, and May 30, 2016, 177 patients were assessed for eligibility. 116 patients were excluded

Figure 2: **Total IgG antibody ELISA responses by treatment regimen**
(A) Kinetics of ID93-specific total IgG responder rate over the study period for each group. A responder was defined as having a 4-times increase in mean endpoint titre (MEPT) from day 0 (baseline). The proportion of responders is plotted for each study day. Vaccine injections were administered on study days 0 and 56, except for the three-injection dosing regimen of the 2 µg ID93+5 µg GLA-SE group, who were vaccinated on study days 0, 28, and 56. Dashed vertical lines indicate days on which patients received vaccine doses. (B) Distribution of MEPT by study participant. Superimposed box plots are data from all participants (midline=median; upper and lower extents=25th and 75th percentiles). Whiskers extend to the most extreme values that are no more than 1·5 times the IQR or to data extremes if no value meets this criterion. Grey lines represent individual participant responses. (C) Kinetics of antigen-specific total immunoglobulin antibody endpoint titres over the study period for participants in cohort 1 (2 µg ID93+2 µg GLA-SE administered on days 0 and 56). ID93+GLA-SE vaccine recipients are shown in green and saline placebo recipients (all cohorts combined) are shown in blue.


A
2 μg ID93 + 2 μg GLA-SE (×2)
10 μg ID93 + 2 μg GLA-SE (×2)
2 μg ID93 + 5 μg GLA-SE (×2)
2 μg ID93 + 5 μg GLA-SE (×3)
Placebo
CD4 T cells (%) producing 2 or more cytokines (%)
4
2
1
0·5
0·1
0·05
≤0·025
0 14 28 56 70 224
0 14 28 42 56 70 224
B
0 14 28 56 70 84 224
Study day
C
Rv1813
0·4238
0·0137
0·0029
0·0039
0·0312
0·0059
0·7334
Rv2608
0·0171
0·0134
0·0002
0·0002
0·0010
0·0002
0·0269
CD4 T cells (%)
3
2
1
0·20
0·15
0·10
0·05
0
Day 0
Day 14
Day 56
Day 70
Day 224
Rv3619
0·0098
0·0923
0·0010
0·0046
0·0010
0·0029
0·0049
Rv3620
0·2334
0·2661
0·0002
0·0015
0·0537
0·0010
0·0640
IFN-γ
IL-2
TNF
Degree of T cell differentiation

(87 screening failure, 29 still in screening at close of enrolment; figure 1). 61 patients were enrolled and randomly assigned to receive 2 μg ID93 + 2 μg GLA-SE on days 0 and 56 (n=15, cohort 1); 10 μg ID93 + 2 μg GLA-SE on days 0 and 56 (n=5, cohort 2); 2 μg ID93 + 5 μg GLA-SE on days 0 and 56 with placebo on day 28 (n=15, cohort 3); 2 μg ID93 + 5 μg on days 0, 28, and 56 (n=14, cohort 3); or placebo on days 0 and 56 (n=5, cohort 1; n=2, cohort 2), or on days 0, 28, and 56 (n=5, cohort 3).

One participant did not receive a study injection in cohort 3 and two participants in cohort 1 received only one of two scheduled injections. In cohort 3, three participants received only one injection and one participant received two of three scheduled injections. Eight patients withdrew consent, one patient was lost to follow-up, and one patient died before the end of the study. 53 patients completed the study and were eligible for inclusion in the per-protocol analysis for immunogenicity, although not all samples were available at all timepoints. 60 patients had at least one dose of study medication and were included in the safety analysis. Age and body-mass index were distributed similarly across the cohorts. More women than men were randomly assigned into cohorts 1 and 2, and more men than women were randomly assigned to cohort 3. More demographic characteristics are presented in table 2.

60 participants received at least one study injection and were included in the analysis of the primary outcome of safety. ID93 + GLA-SE appeared to be safe and well tolerated across all dose regimens tested. The most frequent local AEs were injection site pain, induration, and erythema. Systemic AEs consisted of single instances of arthralgia, fatigue, and myalgia (table 3). There was a significant difference in reactogenicity across dosing schedules and doses, with injection site pain occurring more frequently in those receiving two injections of vaccine at any dose than in those receiving placebo, and erythema occurring more frequently in those receiving (2×) 2 μg + 5 μg than in those receiving placebo. The most frequent safety laboratory abnormality was increased alanine aminotransferase, followed by decreased white blood cells, and increased white blood cells (table 3). Most AEs were self-limiting and resolved without treatment.

Three grade 3 AEs related to vaccination were observed, including: transient increased white blood cells in one participant in the (3×) 2 μg + 5 μg group; and erythema (>10 cm) and induration events in one other participant in the (3×) 2 μg + 5 μg group (counted as two events), which occurred after the second injection and were considered to be related to the vaccine. The participant who had erythema and induration did not receive a third injection. Two vaccine-unrelated SAEs were reported in the placebo group: one participant diagnosed with carcinoma of unknown origin died with a clinical picture consistent with rapidly progressive cerebral herniation, and one participant was admitted to hospital for delirium tremens.

Baseline ID93-specific IgG responses were generally low and remained low in placebo recipients. After vaccination, participants in all ID93 + GLA-SE regimen groups developed a robust ID93-specific antibody response, with similar total IgG mean endpoint titre (MEPT) and response rates in each regimen (figure 2, appendix p 7). Peak IgG responses were observed after the second injection with little or no further increase after the third injection in the group receiving three doses of 2 μg ID93 + 5 μg GLA-SE in cohort 3. Response rates were 100% for all vaccine-containing regimens at 2 weeks after the second injection, and remained at 100% for 6 months after the final injection, compared with 0% in the placebo group (FWER p<0·0001). Antibody responses were predominantly IgG1 and IgG3, and targeted all four ID93 component antigens, with Rv1813 eliciting the highest MEPT (figure 2C).

Baseline and placebo CD4 T-cell responses to ID93 antigens varied among individuals, with a low median response magnitude in PBMC and whole blood (figure 3). After the first dose of vaccination, median response magnitude increased in all vaccine groups (figure 3). In PBMC, day 70 median frequency of ID93-specific CD4 T cells was higher in the (2×) 2 μg ID93 + 2 μg GLA-SE group (FWER p=0·025) and the (2×) 2 μg ID93 + 5 μg GLA-SE group (FWER p=0·0027) than in those who received placebo (appendix pp 7–8). Similar results were observed in whole blood (FWER p=0·0045 for the (2 ×) 2 μg ID93 + 2 μg GLA-SE group and FWER p=0·0061 for the (2 ×) 2 μg ID93 + 5 μg GLA-SE group). ID93-specific CD4 responses were detected in

Figure 3: **Antigen-specific CD4 T-cell responses in stimulated cryopreserved peripheral blood mononuclear cells and fresh whole blood**
Frequencies of ID93-specific CD4 T cells that were positive for at least two immune markers by intracellular cytokine staining assay in (A) peripheral blood mononuclear cells and (B) whole blood. Vaccine injections were administered on study days 0 and 56, except for the three-injection dosing regimen of the 2 μg ID93 + 5 μg GLA-SE group, who were vaccinated on study days 0, 28, and 56. Dashed vertical lines indicate days of drug administration. Results shown are after subtraction of immune marker-expressing CD4 T cells in the unstimulated control sample. Superimposed box plots are based upon data from all participants (midline=median; extents=25th and 75th percentiles). Whiskers extend to the most extreme values that are no more than 1·5 times the IQR or to the data extremes if no value meets this criterion. Grey lines represent individual participant responses. (C) Antigen-specific CD4 T-cell responses in patients given 2 μg ID93 + 5 μg GLA-SE on days 0 and 56. Coexpression of IFNγ, IL-2, or TNF was measured by intracellular cytokine staining assay after 12 h of stimulation of whole blood with specific peptide pools or recombinant ID93 protein. The order of cytokine coexpressing subsets was arranged to represent increasing CD4 T-cell differentiation. The horizontal lines represent medians, the boxes represent IQR, and the whiskers represent the full range. Durability of response of each subset of CD4 T-cell population was measured by Wilcoxon matched-pairs signed rank test comparing day 224 response with pre-vaccination response. p values shown are uncorrected for multiple comparisons. Results shown are after subtraction of cytokine production in the unstimulated control. IFNγ=interferon-γ. IL=interleukin. TNF=tumour necrosis factor.

PBMC and whole blood at 6 months after the final study injection (day 224) in all vaccine groups. In whole blood analysis, median day 224 ID93-specific CD4 T-cell frequencies (FWER p=0·0080; figure 3) and response rates (FWER p=0·016; appendix p 7) in the (2×) 2 μg ID93+5 μg GLA-SE group were significantly higher than in the placebo control group.

Positive CD4 T-cell response rates over the study duration tended to be higher in vaccine-recipient groups (PBMC), but were not significantly different from those of placebo (appendix pp 7,12).

In whole blood, median day 70 CD4 T-cell responses in both the (2×) 2 μg ID93 + 2 μg GLA-SE group (FWER p=0·017) and (2×) 2 μg ID93+5 μg GLA-SE group (FWER p<0·001) were higher than in the (2×) 10 μg ID93+2 μg GLA-SE group; no differences were observed in PBMC. In the two-injection vaccine regimen groups, total CD4 T-cell responses increased from baseline at 14 and 28 days after the first injection in whole blood, but decreased by day 56, and a second injection appeared necessary to boost and sustain response above placebo by day 224. Comparing the two-dose versus three-dose regimens (cohort 3), CD4 T-cell responses were not further increased by a third injection, and although the two-injection regimen trended towards a higher peak median response, this was not significant after multiplicity adjustment.

Vaccine-induced CD4 T-cell responses in both PBMC and whole blood were mostly made up of Rv2608-specific, Rv3619-specific and, to a lesser extent, Rv3620-specific CD4 T cells (figure 3C, appendix pp 13–14). Unlike observed humoral responses, vaccination did not induce substantial CD4 T-cell responses to Rv1813. CD8 T-cell responses were not detected to any antigens, or were very low in magnitude (appendix p 15).

In additional immunogenicity analyses, we compared the magnitude and cytokine coexpression profiles of CD4 T-cell responses induced by ID93+GLA-SE, focusing initially on whole blood responses. The interleukin (IL)-17 response was very low or not detected (data not shown), and was excluded from the analysis. ID93-specific cytokine coexpression profiles were not markedly different between groups, and the memory response at day 224 was mostly made up of CD4 T cells expressing IL-2 alone, CD4 T cells coexpressing tumour necrosis factor (TNF) and IL-2, or polyfunctional cells coexpressing TNF, IL-2, and interferon-γ (IFNγ; appendix p 16). Further analysis of cytokine profiles specific to each of the ID93 antigens was done in the (2×) 2 μg ID93+5 μg GLA-SE group (figure 3C). CD4 T-cell responses targeting Rv2608 were less differentiated (primarily single IL-2$^+$ and IL-2$^+$TNF$^+$) than those CD4 T cells targeting Rv3619 and Rv3620, which also coexpressed IFNγ and mostly contained polyfunctional cells (IFNγ$^+$IL-2$^+$TNF$^+$), whereas TNF$^+$IL-2$^+$ and single IL-2$^+$ subsets were largely absent. These data suggest that CD4 T cells specific for Rv3619 and Rv3620 might be more differentiated than Rv2608-specific CD4 T cells, consistent with an effector phenotype.[27] Responses to Rv1813 were low and the distribution of cytokine-expressing subsets was not easily defined.

To evaluate background response to *M tuberculosis* antigens in participants who had previously been treated for tuberculosis, we used the previously described MTB300 peptide pool consisting of broadly recognised *M tuberculosis* epitopes.[28] To specifically assess responses not induced by the vaccine, epitopes contained in ID93 antigens were omitted from the MTB300 peptide pool. Frequencies of MTB300-specific CD4 T-cell responses in stimulated PBMC varied between participants at baseline, but did not change upon injection in either vaccine or placebo groups (appendix p 17).

We compared functional profiles of ID93-specific and MTB300-specific CD4 T cells in PBMCs at baseline and day 70 using COMPASS[26] to assess the probability of an antigen-specific response for a given combination of immune markers. Heatmaps of all 31 possible combinations of IFNγ, TNF, IL-2, IL-4/IL-13, and CD154 response probabilities for ID93 and MTB300 were generated. Subsets with non-zero probabilities are shown in figure 4. We observed many functional profile subsets responding to MTB300, which is as expected given the many epitopes present in the pool, and these were almost exclusively Th1 responses with few Th2 (IL-4/IL-13) cytokine responses. The most common MTB300-specific profile included a quadruple CD154$^+$TNF$^+$IFNγ$^+$IL-2$^+$ subset and a triple CD154$^+$TNF$^+$IFNγ$^+$ subset, observed at both baseline and day 70 in vaccine and placebo recipients. By contrast, the number of subsets observed for ID93-specific CD4 T cells was higher on day 70 than on day 0. Baseline ID93-specific responses were mostly contained within the same two predominant quadruple CD154$^+$TNF$^+$IFNγ$^+$IL-2$^+$ and triple CD154$^+$TNF$^+$IFNγ$^+$ subsets as MTB300 responses. Vaccination augmented these responses and elicited a distinct, triple CD154$^+$TNF$^+$IL-2$^+$ subset. Unlike placebo, ID93+GLA-SE induced multiple double-positive subsets, in addition to cells expressing all five markers.

Comparison of functional subsets specific for each of the ID93 component antigens revealed more subsets elicited by Rv2608 (figure 4). Rv3619 and Rv3620 responses were made up of the two most predominant quadruple-positive and triple-positive subsets that were seen after MTB300 stimulation. Rv2608 seemed to drive the CD154$^+$TNF$^+$IL-2$^+$ response, which was more prevalent among ID93-specific than MTB300-specific responses. This analysis indicates that ID93 constituents elicit T-cell response profiles that enhance pre-existing CD4 T-cell functional subsets that persist after tuberculosis disease and treatment.

We also analysed memory phenotypes of ID93-specifc CD4 T-cell responses in whole blood before and after vaccination. ID93-specific CD4 T cells primarily

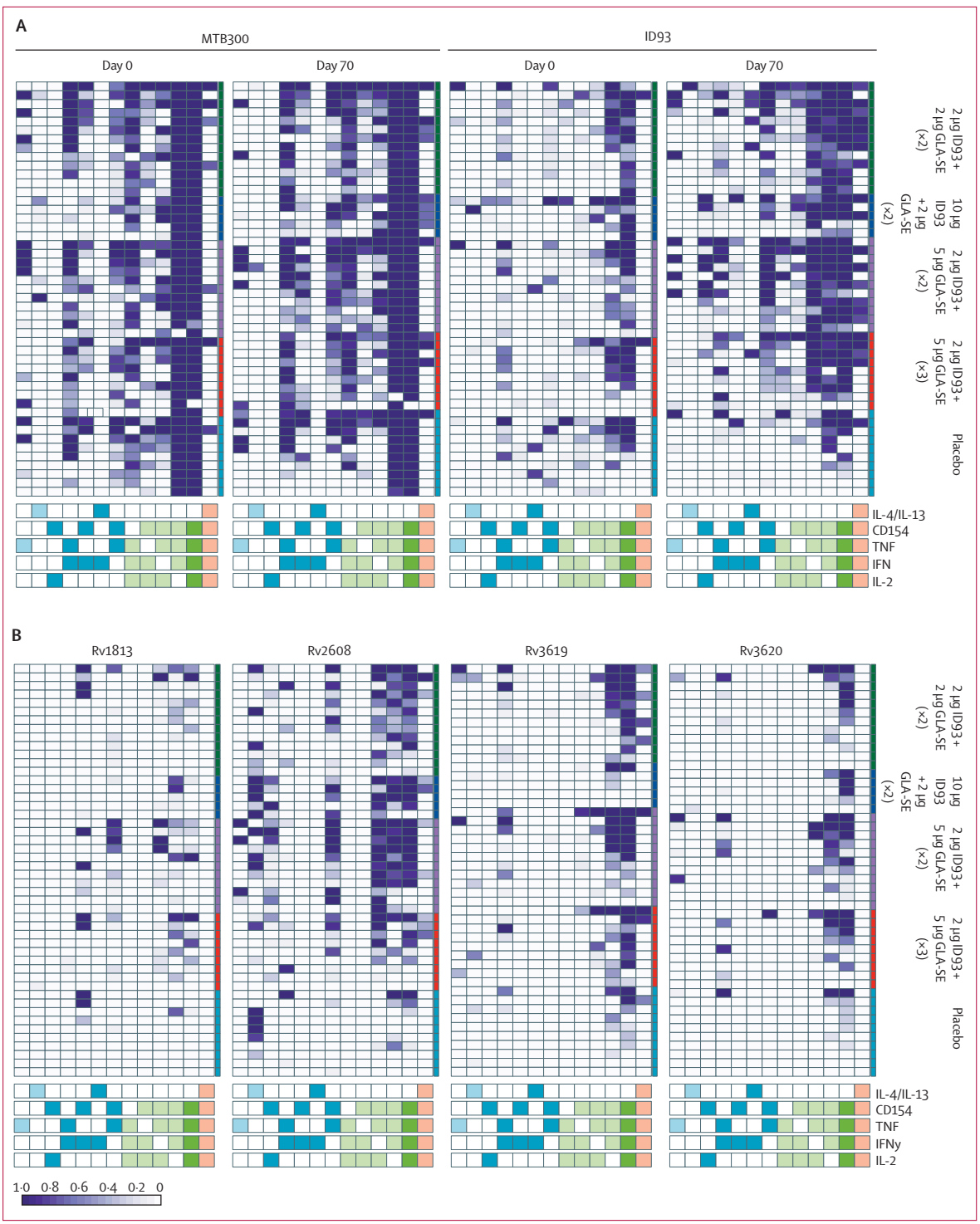


Figure 4: **Phenotypic profiles of vaccine and non-vaccine antigen-specific CD4 T-cell responses in stimulated peripheral blood mononuclear cells**
Stacked COMPASS heatmaps display probability estimates for individual CD4 T-cell responses at (A) days 0 and 70 (2 weeks after the last injection) for MTB300 peptide pool (lacking ID93 epitopes) or ID93 stimulation; and (B) day 70 for each ID93 component antigen. Columns correspond to the cellular subsets evaluated, and are colour-coded by the immune marker they express (white=off; shaded=on) and grouped by number of coexpressing markers in order of increasing polyfunctionality. Rows represent individual participants grouped by cohort. Each heatmap cell shows the probability estimated by COMPASS that the observed response is antigen-specific for the corresponding study day (row) and cell subset (column), where probability is colour-coded from white (zero or background) to purple (one or certainty for antigen specificity). Only subsets with data are included. IFNγ=interferon-γ. IL=interleukin. TNF=tumour necrosis factor.

expressed the CD45RA$^-$CCR7$^+$ central memory or CD45RA$^-$CCR7$^-$ effector memory phenotype (appendix p 18). No marked differences in these phenotypes were observed between different doses and schedules of ID93 + GLA-SE, although cytokine-expressing CD4 T cells in placebo recipients appeared to have the lowest proportions of CD45RA$^-$CCR7$^+$ central memory cells.

Discussion

ID93 + GLA-SE vaccination in adults with previously treated tuberculosis had an acceptable safety profile, and was well tolerated and immunogenic. No evidence for immune suppression or post-vaccination reactions suggestive of local, regional, or distant Koch phenomena were observed. Injection site reactions to ID93 + GLA-SE most frequently included pain, induration, or erythema, and were generally mild, even after three administrations. Few transient systemic reactions were reported, including single instances of arthralgia, fatigue, and myalgia. At the same dose of adjuvant (2 μg), participants receiving a higher dose of ID93 (10 μg) reported a higher rate of injection site pain than did those receiving a lower dose of ID93 (2 μg), whereas those who received a lower dose of the adjuvant GLA-SE (2 μg) reported a lower rate of erythema than those receiving a higher dose (5 μg). However, group sizes were small and definitive conclusions cannot be drawn about dose-related differences in reactogenicity. One participant had grade 3 erythema and induration after the second injection and did not receive a third study injection. Although infrequent in this study, severe erythema and swelling at the injection site were previously observed after vaccination of patients receiving tuberculosis treatment in a study of the protein plus adjuvant vaccine M72/AS01E.[15] Larger studies in this patient population are needed to establish the rate of large injection site reactions following ID93 + GLA-SE vaccination and the extent to which such reactions limit administration of all vaccine doses.

Robust and durable ID93-specific antibody responses were seen in all doses and regimens, with similar peaks after second dose and 6-month mean titres. Antibody responses were elicited to all four ID93 constituents and were predominantly composed of IgG1 and IgG3 subclasses, suggestive of strong MHC class II T-cell help. Additionally, ID93 + GLA-SE vaccination induced a robust, multifunctional CD4 T-cell response that peaked after the second vaccination and persisted for the 6-month study duration. Response magnitudes were not further enhanced by a third injection. Maintenance of ID93-specific CD4 T-cell responses exceeding those of the placebo group was seen only in the (2×) 2 μg ID93 + 5 μg GLA-SE regimen. The acceptable safety profile and the durability of specific CD4 T-cell and antibody responses support further investigation of this vaccine for the prevention of recurrent tuberculosis. The two-injection 2 μg ID93 + 5 μg GLA-SE regimen is recommended for dose sparing as no differences in immunogenicity were observed for increased dose or number of injections. In contrast to humoral responses, which targeted all four vaccine antigens, vaccination did not elicit CD4 T-cell responses to Rv1813 as previously observed.[22,23] Further studies would be needed to understand the mechanisms underlying this observation.

We measured CD4 T-cell responses in both fresh whole blood and cryopreserved PBMC specimens to inform future studies, including larger efficacy trials in which PBMC might provide logistical advantages for biobanking, processing, and targeted post-hoc analysis purposes. Although both methods gave similar results, any conclusions about responses to vaccine compared with placebo, immunodominant antigens, and Th1 cytokine profile are limited by the small group sizes.

Even though study participants had only recently completed treatment for tuberculosis, vaccination elicited polyfunctional CD4 T cells[29] targeting multiple antigens with diverse functional profiles. The Esx family antigens (Rv3619, Rv3620) induced a profile associated with a more differentiated phenotype, whereas the PE/PPE family antigen (Rv2608) induced a less differentiated profile. Vaccine-induced frequencies of antigen-specific cells are similar to those observed in a previous study[22] of ID93 + GLA-SE in latently infected individuals (QuantiFERON-positive), although baseline responses were higher in participants who had recently completed treatment for active tuberculosis.[22] Whether the differentiation or functionality of ID93-specific Th1 cytokine-expressing CD4 T cells will influence efficacy of the vaccine is not known. However, we propose that a vaccine-induced CD4 T-cell response with a broad functional profile is more likely than a narrow response to include protective CD4 T cells. Additionally, induction of T-cell and antibody responses that target multiple antigens expressed by *M tuberculosis* at different stages of infection is likely to be a beneficial characteristic of an effective tuberculosis vaccine. These data therefore provide a strong rationale for further testing of ID93 + GLA-SE.

To characterise the pre-existing, underlying non-vaccine *M tuberculosis*-specific response, and to compare this with the profile of ID93 + GLA-SE-induced responses, we used the MTB300 megapool without ID93 antigens. T-cell responses to MTB300 were not affected by vaccination, suggesting that this megapool could be used in future studies to monitor non-vaccine *M tuberculosis*-specific responses during treatment. COMPASS revealed vaccine-induced CD4 T-cell subsets with unique and overlapping profiles, compared with pre-existing *M tuberculosis*-specific responses.

Strengths of the study include the randomised, double-blind, placebo-controlled design. Limitations include the small sample size, which—although appropriate for a phase 2 trial—does not allow definitive conclusions to be drawn about dose-related differences in reactogenicity. Furthermore, the immediate generalisability of these findings is limited to adjunct vaccination at the end

of a standard course of tuberculosis therapy. Our study assessed only patients treated for drug-sensitive tuberculosis, but ID93+GLA-SE vaccination might be useful earlier, alongside standard and novel drug regimens for both drug-sensitive tuberculosis and drug-resistant tuberculosis, to reduce recurrence rates and allow treatment shortening or simplification. Such benefits of adjunct vaccination might be specific to the treatment regimen. Adjunct therapeutic vaccination might have more potential to improve treatment outcomes for drug-resistant tuberculosis, in which treatment duration, complexity, and toxicity are typically greater than for drug-sensitive tuberculosis. Although vaccine efficacy would be expected to be agnostic to drug sensitivity profile, the safety, immunogenicity, and efficacy of adjunct therapeutic vaccines such as ID93+GLA-SE would also need to be tested iteratively in patients with drug-resistant tuberculosis. However, the scope for benefit of therapeutic vaccination specifically for patients with drug-resistant tuberculosis is difficult to predict accurately, given rapid improvements in treatment outcomes in some trials.[30]

Overall, the data show an acceptable safety profile, provide encouraging evidence of immunogenicity, help in the selection of a regimen of the ID93+GLA-SE vaccine in patients with treated tuberculosis, and provide a strong rationale for further testing of the vaccine. Future clinical trials of ID93+GLA-SE should test the efficacy of post-treatment ID93+GLA-SE vaccination for the prevention of recurrent drug-sensitive tuberculosis, or explore the safety and potential of ID93+GLA-SE to improve therapeutic outcomes when administered during treatment. Our data also support the suitability of ID93+GLA-SE for further evaluation as a preventive vaccine in *M tuberculosis*-infected populations, a role that will be crucial for interruption of tuberculosis transmission and the success of global tuberculosis control efforts.

Contributors

AP-N, TAD, AKKL, L-GB, GW, AD, SGR, RNC, TJS, and MH designed the study. AKKL, MH, L-GB, AD, MT, HG, and JS recruited cohorts and evaluated participant safety. CLA and AS provided custom peptide pool reagents. JV, TAR, TDR, SM, NDP, NB, AGL, AT, and GW collected data used in the experiments. TAD, AF-G, AP-N, AKKL, RNC, TJS, and MH analysed the data and contributed to the interpretation of results. JA, AF, and ZKS provided clinical operations and regulatory support. TAD, AKKL, AP-N, TJS, RNC, and MH wrote the first draft. All authors had full access to the data, and reviewed, revised and gave final approval of the manuscript before submission.

Declaration of interests

SGR reports grants from the Infectious Disease Research Institute (IDRI), Seattle, WA, USA, during the conduct of the study; licence revenue for the vaccine to IDRI from Quratis, outside the submitted work; and a patent issued from the Wellcome Trust for the vaccine (patent number US8486414B2). MH reports an institutional clinical trial grant from the Wellcome Trust via IDRI to the University of Cape Town, during the conduct of the study. The other authors declare no competing interests.

Acknowledgments

We would like to thank Steve DeRosa for helping with the PBMC ICS panel design and analysis.

Date sharing

Upon publication, qualified researchers can contact the corresponding author to obtain specific de-identified clinical trial data with the permission of the sponsor. In addition, the study protocol (see appendix p 19) and statistical analysis plan will be shared.

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